THE JOURNAL OF INVESTIGATIVE DERMATOLOGY

Vol. 50, No. 2
Printed in U.S.A.

SUCTION BLISTER DEVICE FOR SEPARATION OF VIABLE EPIDERMIS FROM DERMIS*

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There is need for technic by which epidermis can be separated from the dermis by purely mechanical forces avoiding chemical or thermal damage. Of the *in vitro* separation technics, Van Scott's stretch method (1) and its modifications (2, 3) have reached widest application. They require, however, comparatively large surgical or necropsy specimens and have the disadvantage that the epidermis, scraped off with a scalpel, may lack the basal cell layer (4).

As with unidirectional stretching, dermal-epidermal separation has been achieved by the application of pressure gradients causing multidirectional distension. As long ago as 1900 Weidenfeld (5) reported that subepidermal blisters could be produced when hydrostatic water pressure was mediated on the dermal side of necropsy specimens of human skin. In 1950 Blank and Miller (6) published a reversal of this method. They applied a forceful suction on the epidermal side of immersed necropsy skin pieces tightly wired across a vacuum thistle. In the hands of Burbach (7) this method yielded inconsistent results.

In vivo separation of the complete human epidermis by suction was accomplished in 1964 (8). Comparatively low suction pressures for prolonged periods succeeded in consistently raising subepidermal blisters.

A literature search revealed that Unna had expressed the use of pressure forces for *in vivo* removal of the epidermis in 1878 (9). In a study of cantharidin blistering he mentioned that blisters can be produced by "trockenes Schröpfen", a certain kind of dry cupping of the intact skin. Apparently Unna did not develop this, not even mentioning it in his review of blister formation (10).

Presented at the Twenty-eighth Annual Meeting of the Society for Investigative Dermatology, June 18–20, 1967, Atlantic City, New Jersey.

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† This study forms a part of the author's thesis and was made under supervision of T. Putkonen, M.D., M.P.H. and K. K. Mustakallio, M.D.

For suction blistering Kiistala and Mustakallio (8) initially used a conventional capillary resistance meter, by which blisters could be raised within two hours by a pressure of 200 mm Hg below the atmospheric pressure. By employing a slightly different suction cup Bielický (11) had attempted to quantitate the Nikolsky phenomenon, but never was able to produce suction blisters on healthy human skin. He did, however, work with too short suction times (up to 30 min.) and applied too forceful a suction (500 mm Hg), the latter resulting in bruising.

After comparing several types and several sizes of suction cups designed for measuring capillary resistance (12, 13, 14, 15) or intraocular pressure (16, 17), the present author came to the conclusion improvements were needed. Hence, a special suction blister device was developed. This enables production of subepidermal blisters of preselected number and size with a simultaneous general decrease of unnecessary suction trauma. This suction blistering has been extended to the furry animal skin and stratified epithelium.

METHOD AND APPLICATIONS

In vivo blistering can be produced by pressure differences ranging from about one hundred to several hundred millimeters of mercury and by cups of a few millimeters to several centimeters in diameter. Strong suction forces of long duration damage the skin. In order to provide suction blisters of good quality, i.e. free of unnecessary trauma, only low pressures can be used. In spite of these low pressures, the blistering produced by the older cups could still be followed by visible trauma as shown by petechiae, persistent flush or edema of the area as well as by histological tearing damage.

In the construction of a device for atraumatic blistering the main problem was to avoid too deep an impression of the cup edge, excessive sliding in and overstretching of the skin. First, the cup edge was remodeled to a comparatively broad flange and was equipped with several shallow grooves. Using this type of contact with the skin, undue impressions could be prevented and an increased air-proofness acquired. During most suctions, a properly shaped bulge of the sucked skin also resulted. However, in regions where the skin is slack and free to slip over the underlying

FIG. 1. The suction blister device, Dermovac®. The three separate parts of the suction cup are presented at bottom left, lying upside down. For use the flange-piece is screwed to the chamber-piece. The adapter plate can be placed inside if required. The suction cup ready for use is shown at bottom right. It is coupled via a stop-cock and a cone-connector to a polyethylene tubing leading to the vacuum pump.

tissue, its resiliency could still cause an excessive filling of the cup despite the grooved cup flange. This adverse phenomenon is exaggerated with an increase of the inner diameter of the cup.

As a rule, larger cups adhere better to the skin than do smaller cups, which is particularly evident with low suction forces. They are also more practical for large samples. However, the displacement of the sucked tissues and the danger of overdistension are greater. In addition, several body regions with rounded outer contours or with bony tissues closely underneath restrict their use. Thus smaller suction cups are also needed.

Most of the properties of human skin could be placed under reasonable control when the broad and grooved flange was combined with the use of different sizes of interchangeable flange pieces. A final improvement was obtained when a perforated, concave diaphragm was placed inside the suction cup as a mould to check the bulging skin. A properly shaped bulge was empirically determined from selected suctions which had resulted in optimally atraumatic blisters. If the perforated diaphragm plate was made plane, the cup did not adhere well to the skin. The use of concave diaphragms across the cup opening inhibited the still existing possibility of an excessive slipping-in of the outside skin into the cup. In addition, by varying the number and size of the round holes in the diaphragm, standard blisters could be raised at will. These improvements are the basis of the construction of the suction blister device Dermovac.*

The Suction Device Dermovac

The suction blister device Dermovac is shown in Fig. 1. It consists of a suction cup and of a separate hand pump with an aneroid gauge.

The suction cup, made of transparent plexiglass, is composed of three separate parts: a flange-piece, a chamber-piece and an adapter plate, the latter being adjustable inside the cup as a diaphragm to prevent excessive bulging.

On the skin side of the flange-piece, which serves as the cup border, are concentric rounded grooves each approximately 1 mm in depth. The flange-pieces, varying in size from 15 mm to 50 mm in inner diameter, are interchangeable and can all be screwed to the standard chamber-piece. A mounted gasket ring of polyethylene ensures an air-proof linkage between the flange-pieces and the chamber-piece.

The chamber-piece, on the other hand, leads via a stop-cock and a cone connector to the tubing of the pump device. When the pressure maintains the desired level, the pump tubing can be released after closure of the stop-cock (Fig. 2) and the pump used for separate suctions. For most purposes, however, it is more useful to have the

* Dermovac® is available from Instrumentarium, Helsinki, Finland.

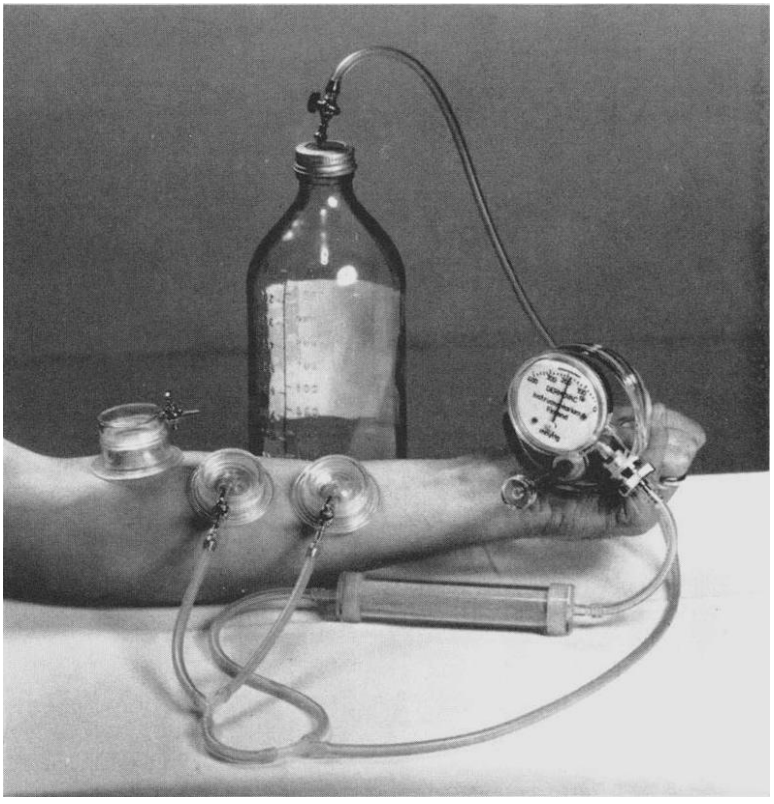

FIG. 2. Three suction cups attached by suction to the forearm. For additional support short strips of adhesive tape running over the flat cup margins may be also used. The cup on the left is disconnected from the pump device. The remaining cups demonstrate simultaneous suction mediated by T-tubes leading to a 1000 cc vacuum stabilizer and to the pump device. Note the filter fitted in front of the pump device to prevent moisture and small particles from reaching its interior.

pump connected for the entire suction period. The blistering process can be visually followed through the transparent roof of the chamber-piece.

The adapter plate is a concave plexiglass mould and is placed as a diaphragm inside a corresponding flange-piece (Figs. 1, 2, 5). The adapter plates have been perforated by either one central or by multiple round holes from one to several millimeters in diameter (Figs. 1, 2, 5), determining the number and size of the suction blisters (Figs. 4, 5).

The pump device consists of the manually worked pump, an aneroid gauge, an air lock operated by a spring, a valve by which the pressure may be regulated at will and the outlet to the tubing. All these are mounted in plexiglass. The aneroid meter is scaled for suctions from 0 to 400 mm Hg below the atmospheric pressure. For in vitro experiments a meter scaled up to 650 mm Hg may be utilized. By coupling a large vacuum container (Fig. 2) the effect of incidental air leaking may be overcome. For periodic calibration of the gauge or for special studies a conventional mercury or water manometer can be easily coupled.

Instructions for the Use of the Suction Device

The size of the suction cup to be used depends on the quantity of epidermis or blister fluid needed, but also on the outer contour, thickness and resiliency of the skin region selected. For large samples the 25 mm suction cup is useful for the cubital fossa, mid-volar forearm and the trunk skin. This cup size is also the largest applicable to the skin of extremities. For the trunk skin suction cups up to 50 mm in diameter can be still used, especially together with adapter plates preventing otherwise painful tensions. 15 to 20 mm suction cups are practical for taut skin (shins) and for baby skin. Miniature cups 5 to 15 mm in diameter have to be used for the skin of small animals and for the mucous membranes.

For cadaver skin suction cups 15 to 70 mm in diameter have all been successfully tested. Comparatively large cups are advisable because a

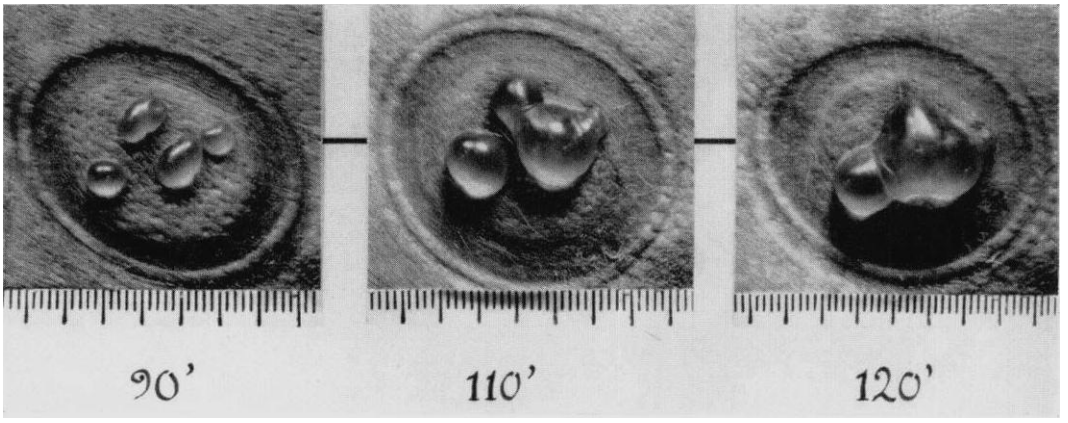

FIG. 3. Without employment of an adapter plate, the suction blisters of varying size gradually coalesce. Of the blisters produced within 90 minutes, three unite 20 minutes and the fourth 30 minutes later to form a single bulla about 20 mm in diameter. The 25 mm suction cup was removed momentarily for photography.

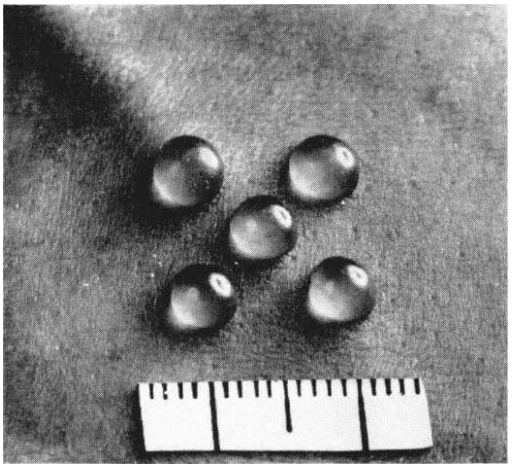

FIG. 4. When an adapter plate with 5 mm-bores was used inside the cup, standard suction blisters, each 4 mm in diameter, could be produced by a suction of 150 mm Hg within 100 minutes.

sufficient suction force to attach the flange-piece airtight upon very dehydrated skin requires not only higher pressures, but larger areas. Generally, gluing of the skin with the flange should be avoided, but may be necessary in dehydrated or hardened skin, as well as in hairy areas.

The suction pressure at the onset should be only slowly increased to the maintenance level, i.e. in about one minute. In placing the cup upon the skin, only gentle manual pressure should be applied. Within a few minutes the cup will adhere to the glabrous skin; in hairy areas an airtight situation is obtained somewhat more slowly. Thereafter, attachment of short strips of adhesive tape over the flat borders of the flange secures the stability of the cup, especially if the test subject is allowed to move freely.

Suction blister times* are not dependent on the suction pressures alone. In old age acceleration of blister formation is observed. Great individual variations and certain regional differences are also evident.

Independent of the size of the cup, suctions of 150 to 200 mm Hg cause initial blistering within three hours on the mid-volar forearm, chest or abdomen. By the use of these comparatively low suction pressures, the dermo-epidermal cleavage begins smoothly in the plane between the basement membrane and the basal plasma membrane of the basal cells, cellular ruptures being mainly confined to the epidermal appendages (18).

The development of the suction blisters can be somewhat hastened by suction pressures of 250 to 350 mm Hg, at the expense, however, of the increased tendency of trauma formation. By still stronger suctions painful stretching and bruising occur regularly.

The adapter plate accelerates the formation of blisters and hinders trauma to some extent. It is necessary when large suction cups are applied on loose skin areas. Moreover, without an adapter plate the blisters appear, grow and coalesce in a haphazard manner (Fig. 3), and a suction bulla of standard size, corresponding to the inner diameter of the cup, is obtained only when the epidermal separation has reached the inner circumference of the flange. With proper choice of an adapter plate, the number and size of suction blisters can be determined at will (Figs. 4 and 5). The smallest standard blisters, developing between the epidermal appendages (Fig. 5), are particularly suited for

* Suction blister time denotes the period of suction from the onset until the first vesicles become visible.

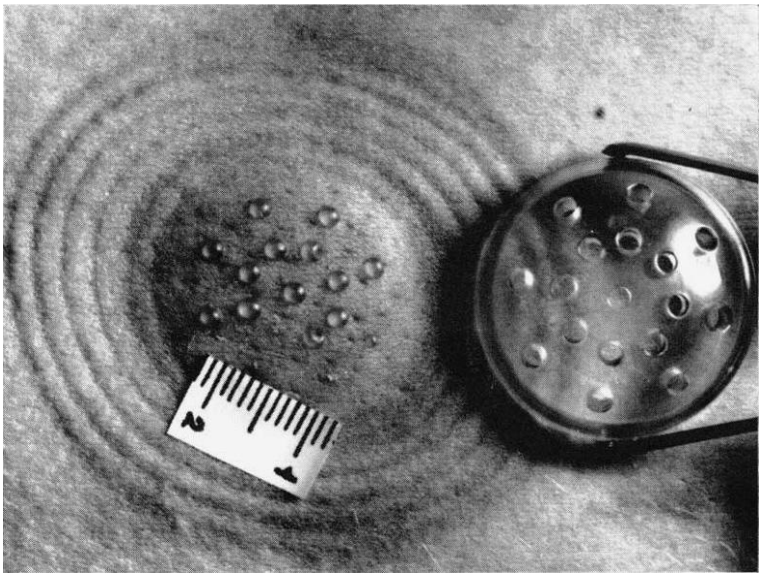

FIG. 5. Suction vesicles produced within 60 minutes, when an adapter plate with 1.5 mm-bores and a suction of 150 mm Hg was employed. The picture taken immediately after the cup was released shows the gentle impressions of the grooved flange. Two of the vesicles formed show an indentation due to an anchoring follicle.

studies where both epidermal and dermal trauma should be at minimum.

The fate of a suction blister depends on its size. Less than one millimeter vesicles disappear within some minutes. Larger blisters do not vanish spontaneously after the release of suction but become somewhat flaccid with time. When sheltered, for instance by the suction cup fixed with adhesive tape, the suction blisters remain unruptured at least for one week. The initially water-clear and acellular (19) blister fluid turns within a few days to an amber-color and contains cells.

Investigative Applications of Suction Blistering

In vivo separation of full-thickness viable epidermis by suction has versatile applications. The sheets of epidermis, uncontaminated by fibroblasts and other dermal cells, provide material for culture of epidermal cells (20). The suction separated epidermis might contribute to the studies in organ culture of mutual interactions of epidermal and dermal components in the formation of the basement membrane material. Future studies will also show, whether or not the epidermal cells of suction blister roofs are adequate for immunological testing and for transplantation.

The suction blister roof suits several kinds of biochemical studies of epidermis (21, 22, 23, 24), for quantitation of epidermal bacteria (25) and for *in vitro* studies of epidermal permeability (26).

Not only the epidermis but also certain stratified mucous epithelia can be separated by suction in living human subjects (Fig. 6). Pure samples of oral epithelium may thus provide material for biochemical comparison with the epidermis.

The diseased human skin does not always respond with blistering to suction. In acute eczema with the loss of the healthy epidermal barrier, only oozing of fluid through the epidermis occurs. However, from certain skin lesions suction bipsies have been successful, for instance, in cases of lichen ruber planus, basal cell epithelioma and discoid lupus erythematosus (Fig. 7).

The determination of suction blister time in certain bullous diseases might be a quantitative substitute for the Nikolsky phenomenon. In our preliminary studies suction times down to as low as two to five minutes were obtained on the erythematous skin of some patients with pemphigus foliaceus or pemphigus erythematosus by suctions of 200 mm Hg. In skin areas near spontaneous lesions of patients with pemphigus vulgaris, Hailey-Hailey, bullous pemphigoid, erythema multiforme and acute urticaria, the blistering times varied greatly from one patient to another, i.e. from that of the normal skin to as low as 20 minutes (27). On the other hand, in several young adults with dermatitis herpetiformis (27) and in two patients with hereditary epidermolysis bullosa the blistering time was always comparable to that of the healthy skin. The possible value of this test can be further studied, when the individual variations and the effects of sex and age on the suction blister time will be established in large enough series.

Suction blisters can be raised on human skin *in vivo* and also *in vitro,* i.e. on the cadaver skin at room temperature and even at 0° C. The suction time here is generally greatly retarded, to as long

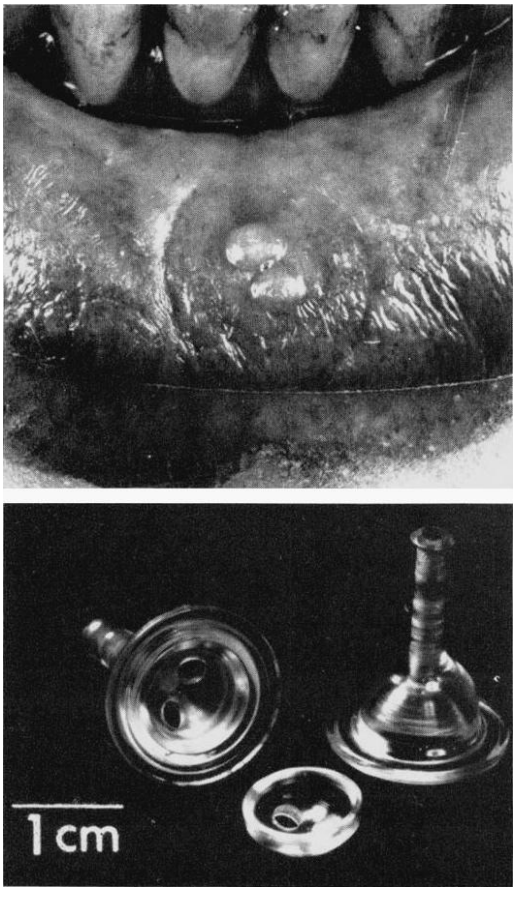

FIG. 6. Two suction blisters, 3 mm in diameter, produced on the buccal epithelium of the lower lip within 80 minutes by a suction of 80 mm Hg. The miniature suction cup with a 7 mm inner-diameter and the adapter plate used are shown below.

as six to 24 hours if suction pressures of 200 mm Hg are used.

Suction detachment of small specimens of epidermis can be achieved also on furry animal skin. After clipping the hair and gluing a small cup to the skin, blistering could be accomplished *in vivo* on adult guinea pigs (Figs. 8 and 9) and on the downy skin of newborn rats.

The blister fluid of minimally traumatized blisters is a water-clear transudate, presumably a molecular filtrate of blood serum and lymph, containing about 2.0 per cent protein and neither red cells nor inflammatory cells. It may serve as a substitute for tissue fluid or lymph (28). The acellularity of the fluid in small fresh blisters is the prerequisite for studies of cellular dynamics in toxic or allergic inflammation (19).

The base of the suction blister functions as a skin window, permits visualization of the capillaries and even of deeper vessels in a comparatively undamaged state (Fig. 10). Also the blood corpuscles can be visualized and their circulation studied. The blister base is well suited for testing of pain producing substances, including plasma kinins, histamine and allergens.

Regeneration of the epidermis can be studied under controlled conditions, because the suction trauma of the dermis is small and of standard nature and the denuded area can be measured.

SUMMARY

Dermo-epidermal separation by application of suction on normal human skin has been accomplished. Subepidermal blisters are regularly raised when low suction pressures are maintained for sufficiently extended periods. Previously used suction cups were all designed for other purposes and caused difficulties in consistent production of suction blisters in a minimally traumatic and standard way.

By construction of a special device, standard suction blisters of preselected size and number can be raised on human skin without discomfort in about two hours by a suction

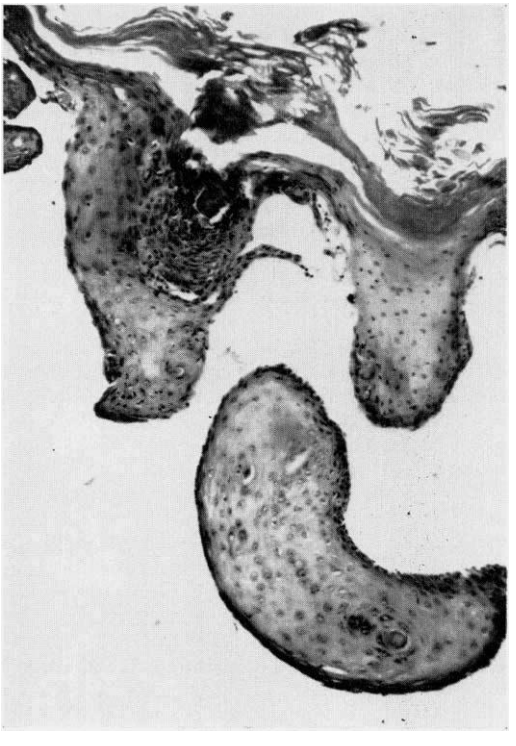

FIG. 7. Suction biopsy of a lesion of discoid erythematosus showing characteristic keratinous plugs.

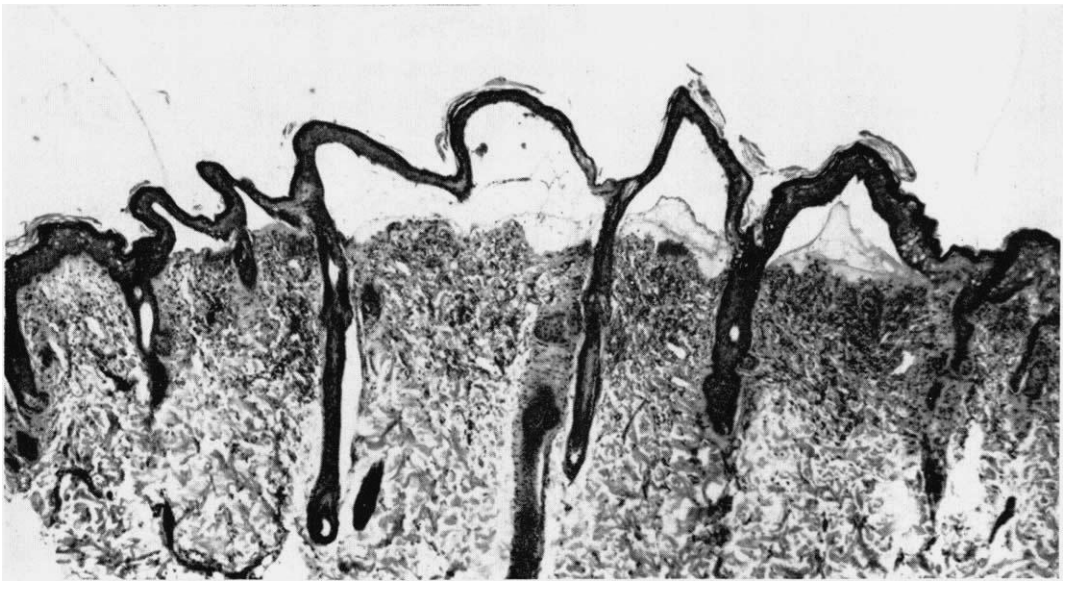

FIG. 8. Histologic appearance of a suction blister produced on clipped abdominal skin of an adult living guinea pig. This section from the blister border shows dermo-epidermal separation of the interfollicular areas. Fibrinous exudate within the cavities and cellular infiltration of the corium are indications of moderate suction trauma in the furry skin. This suction blister of 3 mm in diameter was produced by the miniature suction cup seen in Fig. 6, after gluing of the cup to the skin.

FIG. 9. At higher magnification the separation of the complete epidermis is shown more clearly.

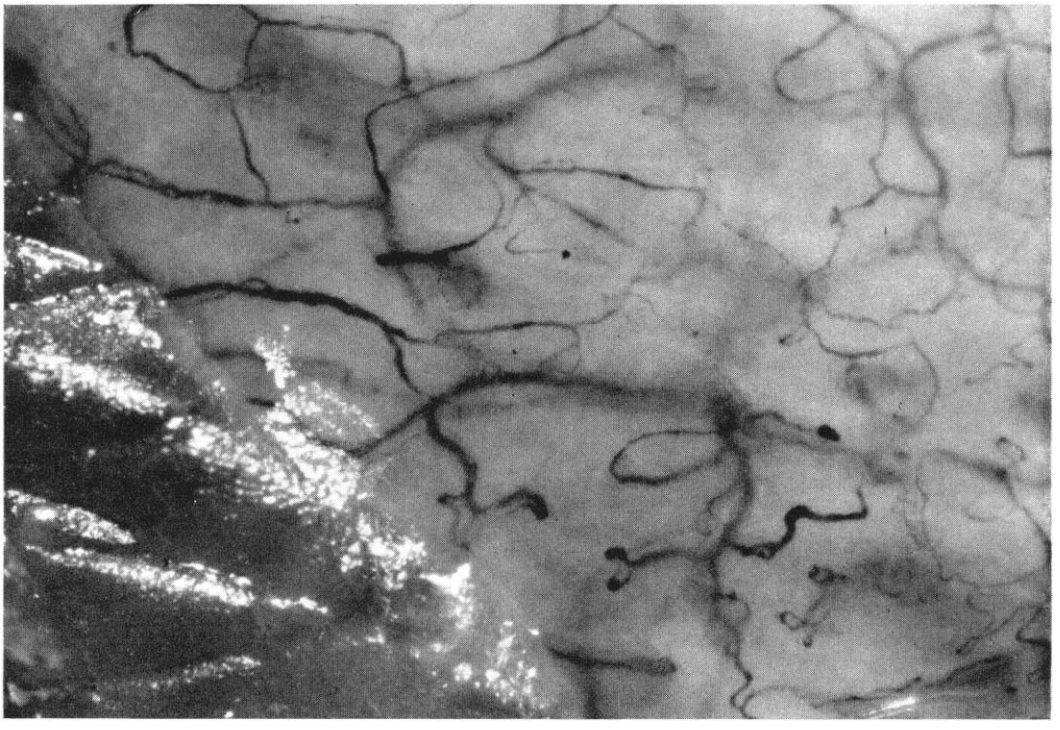

FIG. 10. The base of the suction blister forms an actual skin window. Thus even deeper venous plexus can easily be brought into focus.

of 150 mm Hg. The blister roof consists of viable, full-thickness epidermis; the clear fluid is a non-inflammatory transudate, and there is no resultant scarring.

By this suction device it is also possible to separate the epidermis from corpse skin and certain furry animals, as well as the stratified epithelium of buccal mucosa.

The suction separated epidermis should be useful for tissue culture and biochemical analyses. Some other uses of the suction blister method are suggested.

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